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Evaluation of Reformulated Chemiluminescent DNA Probe (AccuProbe) for Culture Identification of *Mycobacterium kansasii*

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A panel of 104 isolates belonging to the species *Mycobacterium kansasii* and 78 mycobacterial isolates belonging to other species was tested in parallel with the present commercially available DNA probe (AccuProbe; Gen-Probe) and with a new probe just developed by the same manufacturer. While the old version of the probe confirmed the previously reported low sensitivity (only 59% of the *M. kansasii* isolates reacted), the new one was 100% sensitive. Only two non-*M. kansasii* strains, both *M. gastri* isolates, gave false-positive hybridization results.

The availability of commercial DNA probes has greatly contributed to increased levels of mycobacterial identification; thanks to a user-friendly technique, molecular probes even allow less experienced laboratories to correctly and rapidly identify the most frequently isolated mycobacteria.

The DNA probes specific for the *Mycobacterium tuberculosis* complex and the *M. avium* complex (MAC) now play a major role in modern mycobacteriological diagnostics, especially because these organisms are more frequently encountered because of the AIDS pandemic than they were several years ago.

Numerous evaluations (2, 3, 5, 8, 12) have indicated the reliability and accuracy of commercially available DNA probes (Gen-Probe Inc., San Diego, Calif.). A minor flaw, like the failure of probes for *M. avium* and *M. intracellulare* to recognize the so-called MAIX strains, has readily been eliminated with the release of a new probe covering the entire MAC (17).

In a previous extensive study (15), however, we showed an unusually low sensitivity (72.8%) of the AccuProbe for *M. kansasii*, and similar results were reported by others (10, 14); interestingly, all nonhybridizing isolates came from European laboratories. In the precommercialization trial performed in the United States by Gen-Probe, few such strains (5%) had been detected, thus suggesting a nonhomogeneous worldwide distribution (4).

In the study described here the sensitivity and the specificity of an *M. kansasii* DNA probe, reformulated by the manufacturer to overcome the previous limitations, were evaluated in identifying 182 mycobacterial isolates from single patients and were compared with those of the previous version of the probe (AccuProbe for *M. kansasii*; Gen-Probe). The test panel included all the *M. kansasii* strains in our laboratory collection of strains. All the strains had been previously identified by conventional methods (13) by using a wide panel of tests, including all tests most relevant for the identification of this species. The identities of the majority of such strains also had been confirmed by high-performance liquid chromatography of cell wall mycolic acids (1).

Although all *M. kansasii* strains had already been tested with AccuProbe, the present hybridization trial was carried out in parallel with the old and the new versions of the probe. The test was performed according to the manufacturer's specifications (4) on fresh subcultures presenting on solid medium great variability in the extent of growth, from barely visible to fully mature colonies; in no case, however, were cultures that were grown for more than 5 weeks used. Two bacterial lysates were prepared for each culture; they were mixed before the transfer of 100 μl into each of two tubes containing the lyophilized DNA probes to be compared. The light signal emitted was measured with a Leader 50 luminometer (Gen-Probe). The results obtained with both versions of the probes, expressed in relative light units (RLUs), were considered positive when a signal greater than 30,000 RLUs was achieved; when a value ranging from 10,000 to 30,000 RLUs was obtained, the test was repeated.

Of the 182 isolates tested, 104 were identified as *M. kansasii* and 78 were identified as belonging to 61 different mycobacterial species other than *M. kansasii* (Table 1). Forty-three of 104 (41%) *M. kansasii* isolates, including all isolates that had failed to hybridize in our previous evaluation and that had survived since that study (15), did not react with the old version of AccuProbe but gave positive results with the new version.

Only two false-positive reactions were detected, with 2 strains of *M. gastri*, among the 78 isolates belonging to species other than *M. kansasii*. Interestingly, while the two *M. gastri* isolates which unexpectedly hybridized were clinical isolates, a reference strain was clearly negative.

The extent of the signals ranged from 35,717 to 1,066,001 RLUs for positive samples and from 558 to 9,246 RLUs for negative ones (Table 2). Three of about a dozen poorly growing cultures gave borderline results (14,208, 21,752, and 25,788 RLUs, respectively, with the old probe and 22,325, 28,481, and 36,699 RLUs, respectively, with the new one); the same cultures were retested after an additional 1 week of incubation, and clearly positive results were achieved (with 262,598 RLUs being the lowest value). Therefore, a signal ranging from 10,000 to 30,000 RLUs should never be accepted as final because it could conceal a false-negative result attributable to a culture that is not actively growing; in these cases repetition of the test is crucial, preferably after an additional few days of incubation.

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TABLE 1. Mycobacteria tested

Species (no. of isolates)[a]	Source (no. of isolates)[b]	Reference[c]
MAIX	C	
M. abscessus	C	
M. agri	R	ATCC 27406
M. aichiense	R	NCTC 10820
M. alvei	R	CIP 103464
M. asiaticum	C	
M. aurum	R	ATCC 23366
M. austroafricanum	R	ATCC 33464
M. avium (4)	C	
M. bovis	C	
M. bovis BCG	C	
M. branderi	R	ATCC 51789
M. brumae	R	CIP 103465
M. celatum	C	
M. chelonae (2)	C	
M. chitae	R	ATCC 19627
M. chlorophenolicum	R	CIP 104189
M. chubuense	R	NCTC 10819
M. conspicuum	C	
M. diernhoferi	R	ATCC 19340
M. duvalii	R	NCTC 358
M. fallax (2)	C (1), R (1)	CIP 8139
M. farcinogenes	R	NCTC 10955
M. flavescens	C	
M. fortuitum	C	
M. gadium	R	ATCC 27726
M. gastri (3)	C (2), R (1)	ATCC 15754
M. genavense	C	
M. gilvum	R	NCTC 10742
M. gordonae (4)	C	
M. haemophilum	R	ATCC 29548
M. interjectum	C	
M. intermedium	R	DSM 44049
M. intracellulare (2)	C	
M. kansasii (104)	C (92), E (12)	
M. komossense	R	ATCC 33013
M. lentiflavum	C	
M. malmoense	C	
M. marinum (2)	C	
M. mucogenicum	C	
M. neoaurum	R	ATCC 25795
M. nonchromogenicum	C	
M. obuense	R	NCTC 10778
M. parafortuitum	R	ATCC 19686
M. paratuberculosis	R	ATCC 3493
M. porcinum	R	ATCC 33776
M. poriferae	R	ATCC 35087
M. pulveris	C	
M. rhodesiae	R	NCTC 10779
M. scrofulaceum	C	
M. senegalense	R	NCTC 10956
M. shimoidei	C	
M. simiae	C	
M. smegmatis	C	
M. sphagni	R	ATCC 33027
M. szulgai	R	NCTC 10831
M. thermoresistibile	R	ATCC 19527
M. terrae (3)	C	
M. tokaiense	R	NCTC 10821
M. triviale	R	ATCC 23292
M. tuberculosis (4)	C	
M. xenopi	C	

[a] One isolate of each species was tested unless stated otherwise.
[b] C, clinical; E, environmental; R, reference strain. All isolates were from the indicated source unless indicated otherwise.
[c] ATCC, American Type Culture Collection; CIP, Collection de l'Institut Pasteur; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen; NCTC, National Collection of Type Cultures.

TABLE 2. Chemiluminescent hybridization signals of two versions of *M. kansasii* DNA probes with different mycobacterial species

Species (no. of isolates)	RLU (avg ± SD [range])[a]	
	Old probe	New probe
M. kansasii positive with both probes (61)[b]	425,032 ± 199,302 (35,717–677,210)	602,984 ± 277,390 (52,057–1,066,001)
M. kansasii positive only with new probe (43)	1,235 ± 2,116 (558–14,246)	174,134 ± 72,475 (35,841–270,848)
M. gastri (2)[c]	8,923–11,376	82,742–102,839
Mycobacteria other than *M. kansasii* (76)[d]	1,008 ± 309 (701–2,881)	1,753 ± 471 (288–3,561)

[a] The cutoff was 30,000 RLUs.
[b] Data for three isolates which gave borderline RLUs on initial testing are from repeat tests.
[c] Data indicate the actual range of values obtained.
[d] Including the nonhybridizing isolate of *M. gastri.*

In all instances the signal given by the revised probe was superior to that given by the previous version.

The present study demonstrates the excellent sensitivity (100%) and the good specificity (97%) of the reformulated AccuProbe for *M. kansasii*. Unchanged remains the clear-cut distinction of positive and negative results, with a mean positive hybridization signal being about 200 times that of a negative one.

The replacement of the previous probe with the new probe will thus improve the reliability of identification of this species and will perhaps suggest an apparent increase in its prevalence because of the isolation of those isolates which are missed by laboratories which base the identification of mycobacteria only on DNA probe hybridization.

M. kansasii is a potentially pathogenic species most frequently involved in pulmonary tuberculosis-like infections in nonimmunocompromised patients (18); moreover, it ranks third, behind *M. tuberculosis* and MAC, as a cause of disseminated infection in AIDS patients (6); its susceptibility to the majority of antituberculosis drugs, however, makes infections with this organism treatable (7, 11). The presence of different genotypes (19) and of at least two biotypes (16) within the species *M. kansasii* has been reported; a distinction of the clinical significance between such types has not been proven, but a distinction is suggested by our finding of a high degree of prevalence, of the biotype which failed hybridization with AccuProbe, among the isolates of *M. kansasii* obtained from AIDS patients (16).

The identification of 100% of the strains belonging to this species is therefore important, and thus, the improvements to the original probe that have resulted in the DNA probe assessed in the present study appear significant.

The discrepant reactions achieved with two of three *M. gastri* strains remains unexplained. The behavior of such strains with the old probe is peculiar; both strains consistently gave high but clearly negative results (11,376 and 8,923 RLUs, respectively, and, after repetition, 22,046 and 22,521 RLUs, respectively). *M. kansasii* and *M. gastri* share an identical signature sequence within the hypervariable regions of 16S rRNA (9), but the nonhomogeneous behaviors of different *M. gastri* isolates does not seem to support the hypothesis that the new probe is aimed at that region (the manufacturer does not declare the target of the new probe).

The rarity of *M. gastri* isolation (in recent years we detected only one such strain among more than 1,000 mycobacteria other than *M. tuberculosis*, and only a second isolate was obtained from other laboratories) and the easy distinction be-

tween the two species, thanks to the lack of pigmentation of *M. gastri* in comparison with the evident photochromogenicity of *M. kansasii*, emphasize the minor importance of this limitation.

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REFERENCES

1. **Butler, W. R., K. C. Jost, and J. O. Kilburn.** 1991. Identification of mycobacteria by high-performance liquid chromatography. J. Clin. Microbiol. **29:**2468–2472.
2. **Drake, T. A., J. A. Hindler, G. W. Berlin, and D. A. Bruckner.** 1987. Rapid identification of *Mycobacterium avium* complex in culture using DNA probes. J. Clin. Microbiol. **25:**1442–1445.
3. **Ellner, P. D., T. E. Kiehn, R. Cammarata, and M. Hosmer.** 1988. Rapid detection and identification of pathogenic mycobacteria by combining radiometric and nucleic acid probe methods. J. Clin. Microbiol. **26:**1349–1352.
4. **Gen-Probe Inc.** 1993. *Mycobacterium kansasii* culture identification test. Gen-Probe Inc., San Diego, Calif.
5. **Goto, M., S. Oka, K. Okuzumi, S. Kimura, and K. Shimada.** 1991. Evaluation of acridinium-ester-labeled DNA probes for identification of *Mycobacterium tuberculosis* and *Mycobacterium avium-Mycobacterium intracellulare* complex in culture. J. Clin. Microbiol. **29:**2473–2476.
6. **Horsburgh, C. R., Jr., and R. M. Selik.** 1989. The epidemiology of disseminated nontuberculous mycobacterial infection in the acquired immunodeficiency syndrome (AIDS). Am. Rev. Respir. Dis. **139:**4–7.
7. **Inderlied, C. B., and M. Salfinger.** 1995. Antimicrobial agents and susceptibility tests: mycobacteria, p. 1385–1404. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. ASM Press, Washington, D.C.
8. **Kiehn, T. E., and F. F. Edwards.** 1987. Rapid identification using a specific DNA probe of *Mycobacterium avium* complex from patients with acquired immunodeficiency syndrome. J. Clin. Microbiol. **25:**1551–1552.
9. **Kirschner, P., B. Springer, U. Vogel, A. Meier, A. Wrede, M. Kiekenbeck, F. C. Bange, and E. C. Böttger.** 1993. Genotypic identification of mycobacteria by nucleic acid sequence determination: report of a 2-year experience in a clinical laboratory. J. Clin. Microbiol. **31:**2882–2889.
10. **Lebrun, L., F. Espinasse, J. D. Poveda, and V. Vincent Lévy-Frébault.** 1992. Evaluation of nonradioactive DNA probes for identification of mycobacteria. J. Clin. Microbiol. **30:**2476–2478.
11. **Levine, B., and R. E. Chaisson.** 1991. *Mycobacterium kansasii* a cause of treatable pulmonary disease associated with advanced human immunodeficiency virus (HIV) infection. Ann. Intern. Med. **114:**861–868.
12. **Musial, C. E., L. S. Tice, L. Stockman, and G. D. Roberts.** 1988. Identification of mycobacteria from culture using the Gen-Probe rapid diagnostic system for *Mycobacterium avium* complex and *Mycobacterium tuberculosis* complex. J. Clin. Microbiol. **26:**2120–2123.
13. **Nolte, F. S., and B. Metchock.** 1995. *Mycobacterium*, p. 400–437. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. ASM Press, Washington, D.C.
14. **Ross, B. C., K. Jackson, M. Yang, A. Sievers, and B. Dwyer.** 1992. Identification of a genetically distinct subspecies of *Mycobacterium kansasii*. J. Clin. Microbiol. **30:**2930–2933.
15. **Tortoli, E., M. T. Simonetti, C. Lacchini, V. Penati, C. Piersimoni, and V. Morbiducci.** 1994. Evaluation of a commercial DNA probe assay for the identification of *Mycobacterium kansasii*. Eur. J. Clin. Microbiol. Infect. Dis. **13:**264–267.
16. **Tortoli, E., M. T. Simonetti, C. Lacchini, V. Penati, and P. Urbano.** 1994. Tentative evidence of AIDS-associated biotype of *Mycobacterium kansasii*. J. Clin. Microbiol. **32:**1779–1782.
17. **Viljanen, M. K., L. Olkkonen, and M. L. Katila.** 1993. Conventional identification characteristics, mycolate and fatty acid composition, and clinical significance of MAIX AccuProbe-positive isolates of *Mycobacterium avium* complex. J. Clin. Microbiol. **31:**1376–1378.
18. **Wolinsky, E.** 1979. Nontuberculous mycobacteria and associated diseases. Am. Rev. Respir. Dis. **119:**107–159.
19. **Yang, M., B. C. Ross, and B. Dwyer.** 1993. Identification of an insertion sequence-like element in a subspecies of *Mycobacterium kansasii*. J. Clin. Microbiol. **31:**2074–2079.